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Growth potential of three strains of *Listeria monocytogenes* and *Salmonella enterica* in Frescal and semi-hard artisanal Minas microcheeses: Impact of the addition of lactic acid bacteria with antimicrobial activity

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ABSTRACT

This study aimed to determine the growth potential (δ) of *L. monocytogenes* (CLIST 3974, CLIST 3969, and CLIST 4162) and *S. enterica* [*S.* Typhimurium (ATCC SM 14028), *S.* Enteritidis (SM 64), and *S.* Montevideo (SM 129)] in the presence of a pool of lactic acid bacteria (LAB) with antimicrobial activity in Frescal and semi-hard Minas microcheeses. The δ was determined after storing Frescal cheese at 4 and 7 °C for 15 days and the and semi-hard Minas cheese during ripening (22 °C for 22 days). The δ of *L. monocytogenes* was significantly higher in Frescal Minas cheese with no added LAB ($p > 0.05$). On the other hand, in semi-hard cheese inoculated with LAB, inactivation of *L. monocytogenes* was observed. No significant differences were found in the δ of *S. enterica* in Frescal Minas cheese inoculated with LAB at 4 and 7 °C. *S. enterica* SM 14028 and SM 129 could grow in semi-hard cheeses non-inoculated with LAB, while when LAB was inoculated, *S. enterica* was inactivated. The findings of this study indicated that the δ of *L. monocytogenes* strains was more affected in cheeses inoculated with LAB than the δ of *S. enterica*.

1. Introduction

In Brazil, the cheese market is characterized by about 40% artisanal cheeses, and consumption corresponds to 5.9 kg per inhabitant, with growth of 68.8%/year between 2009 and 2014 (Gomes, Pithan, Van Dender, & Zacarchenco, 2017). Artisanal cheesemaking is characterized by traditional techniques from each region and small-scale production (Cardoso & Marin, 2017). Minas cheeses are very popular cheeses produced in the state of Minas Gerais and include the soft, semi-hard, and hard types (Campagnollo et al., 2018).

Frescal Minas cheese is a soft, fresh white Brazilian cheese, traditionally produced by enzymatic coagulation of pasteurized milk with rennet or other appropriate coagulant enzymes, supplemented with lactic acid bacteria cultures (Cunha-Neto et al., 2020). Frescal Minas cheese is characterized as a semi-fat (25–45 g/100g fat in the dry matter), slightly salty, slightly acidic, highly perishable (15–20 days of shelf life) cheese even under refrigeration (Pimentel, Marcolino, Barão, Klososki, & Rosset, 2019). On the other hand, semi-hard Minas cheese is generally produced from raw milk inoculated with commercial rennet and "pingo," a fermented whey that contains LAB obtained from the previous cheese production (Campagnollo et al., 2018). "Pingo" is responsible for developing the sensory characteristics of these cheeses and may also inhibit or inactivate foodborne pathogens (Campagnollo et al., 2018; Kamimura, De Filippis, Sant'Ana, & Ercolini, 2019). Semi-hard Minas cheese is not cooked, presents a thin crust with yellowish color, no cracks, white-yellowish mass, semi-hard consistency, slightly acidic taste, and a marked and spicy flavor (Amarante, 2015). The ripening period of semi-hard Minas cheese is about 22 days at room temperature (Cadavez et al., 2019; Kamimura, De Filippis, et al., 2019).

Regardless of high appreciation and consumption in Brazil, Frescal and semi-hard Minas cheeses can harbor foodborne pathogens (Nunes,

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Table 1
Growth potential (δ) of lactic acid bacteria and strains of L. monocytogenes in Frescal and semi-hard Minas cheeses during refrigerated storage (15 days) and ripening (22 days), respectively.

Storage temperature	Treatments[a,b]	δ (log CFU/g)	
		LAB	LM[c]
Frescal Minas Cheese			
4 °C	LM 3974 + LAB	0.25 ± 0.09^{d}	0.17 ± 0.64^{b}
	LM 3974	3.60 ± 0.71^{a}	3.33 ± 0.14^{a}
	LM 3969 + LAB	0.18 ± 0.28^{d}	1.10 ± 0.65^{b}
	LM 3969	1.93 ± 0.01^{bc}	3.37 ± 0.09^{a}
	LM 4162 + LAB	0.56 ± 0.05^{cd}	0.89 ± 0.23^{b}
	LM 4162	2.96 ± 0.18^{ab}	2.91 ± 0.39^{a}
p-value		0.0007	0.0008
7 °C	LM 3974 + LAB	1.40 ± 0.62^{c}	0.61 ± 0.91^{c}
	LM 3974	3.66 ± 0.01^{a}	4.21 ± 0.29^{a}
	LM 3969 + LAB	0.89 ± 0.20^{c}	1.09 ± 0.64^{bc}
	LM 3969	2.20 ± 0.33^{bc}	4.13 ± 0.10^{a}
	LM 4162 + LAB	1.16 ± 0.06^{c}	0.94 ± 0.60^{c}
	LM 4162	3.26 ± 0.20^{ab}	3.12 ± 0.19^{ab}
p-value		0.0032	0.0012
Semi-hard Minas Cheese			
22 °C	LM 3974 + LAB	1.26 ± 0.57^{a}	−0.30 ± 0.01^{a}
	LM 3974	1.60 ± 0.38^{a}	0.78 ± 0.40^{a}
	LM 3969 + LAB	1.33 ± 0.65^{a}	−1.74 ± 0.01^{a}
	LM 3969	3.47 ± 1.73^{a}	0.58 ± 1.20^{a}
	LM 4162 + LAB	1.35 ± 0.10^{a}	−1.36 ± 0.56^{a}
	LM 4162	2.97 ± 0.32^{a}	1.45 ± 0.48^{a}
p-value		0.1203	0.0766

[a-d]: Means followed by the same superscript letter indicate no significantly difference according to Tukey's test (P ≤ 0.05).
[a] Treatments included cheeses separately inoculated with three different strains of *L. monocytogenes* (LM) (LM 3974, LM 3969 and LM 4162) and added or not with the pool of antimicrobial LAB.
[b] Values expressed as mean ± standard deviation.
[c] Negative δ values indicate inactivation of LM.

Table 2
Growth potential (δ) of lactic acid bacteria (LAB) and strains of Salmonella enterica (SM) in Frescal and semi-hard Minas cheeses during refrigerated storage (15 days) and ripening (22 days), respectively.

Storage temperature	Treatments[a,b]	δ (log CFU/g)	
		LAB	SM[c]
Frescal Minas Cheese			
4 °C	SM 14028 + LAB	0.47 ± 0.06^{a}	−1.54 ± 0.32^{a}
	SM 14028	−0.05 ± 0.01^{a}	−1.82 ± 0.18^{a}
	SM 64 + LAB	0.30 ± 0.01^{a}	−0.50 ± 0.05^{a}
	SM 64	0.44 ± 0.01^{a}	−1.07 ± 0.85^{a}
	SM 129 + LAB	0.68 ± 0.47^{a}	−0.11 ± 0.01^{a}
	SM 129	0.19 ± 0.45^{a}	−1.35 ± 0.50^{a}
p-value		0.6856	0.1344
7 °C	SM 14028 + LAB	1.27 ± 0.32^{a}	−0.30 ± 0.25^{a}
	SM 14028	0.65 ± 0.03^{a}	0.15 ± 0.25^{a}
	SM 64 + LAB	0.65 ± 1.09^{a}	1.05 ± 0.66^{a}
	SM 64	1.82 ± 1.92^{a}	0.79 ± 0.52^{a}
	SM 129 + LAB	1.77 ± 0.55^{a}	0.65 ± 0.10^{a}
	SM 129	4.07 ± 1.00^{a}	−0.05 ± 0.01^{a}
p-value		0.1022	0.1220
Semi-hard Minas Cheese			
22 °C	SM 14028 + LAB	1.38 ± 0.05^{b}	−1.70 ± 0.01^{b}
	SM 14028	1.84 ± 0.61^{b}	0.93 ± 0.01^{a}
	SM 64 + LAB	2.36 ± 0.49^{b}	−0.98 ± 0.01^{ab}
	SM 64	5.06 ± 0.14^{a}	−0.19 ± 0.81^{ab}
	SM 129 + LAB	1.50 ± 0.01^{b}	−2.20 ± 0.54^{b}
	SM 129	1.14 ± 0.24^{b}	0.69 ± 0.00^{a}
p-value		0.0010	0.0173

[a-d]: Means followed by the same superscript letter indicate no significantly difference according to Tukey's test (P ≤ 0.05).
[a] Treatments included cheeses separately inoculated with three different strains of *Salmonella enterica* (SM) (SM 14028, SM 64 and SM 129) and added or not with the pool of antimicrobial LAB.
[b] Values expressed as mean ± standard deviation.
[c] Negative δ values indicate inactivation of SM.

Mota, & Caldas, 2013) as well as be involved in foodborne disease outbreaks (Simeão do Carmo et al., 2002). Cheeses can be contaminated with pathogenic microorganisms through the milk utilized and inadequate practices during production and storage (Paramithions, Drosinos, & Skandamis, 2017). *Listeria monocytogenes* causes listeriosis, a foodborne disease characterized by high mortality (20–40%) and hospitalization (>95%) rates (Buchanan, Gorris, Hayman, Jackson, & Whiting, 2017). Various listeriosis outbreaks have been linked to ready-to-eat foods (Barría et al., 2020; Gérard et al., 2020; Prezzi et al., 2020), particularly unripe cheese or ripened soft cheese made from raw or low-heat-treated milk (EFSA & ECDC, 2018; Martinez-Rios & Dalgaard, 2018). Another pathogen of significant concern is *Salmonella enterica*, the causative agent of salmonellosis (CDC, 2020; WHO, 2020), which has been increasingly linked to gastroenteritis outbreaks due to the consumption of artisanal cheeses (Andretta et al., 2019; Costanzo, Carlotta, Santoro, Clausi, & Casalinuovo, 2020). *S.* Typhimurium, *S.* Enteritidis, and *S.* Montevideo comprise the main serovars implicated in cheeses outbreaks (Lobacz, Kowalik, & Zulewska, 2020; Martínez et al., 2020).

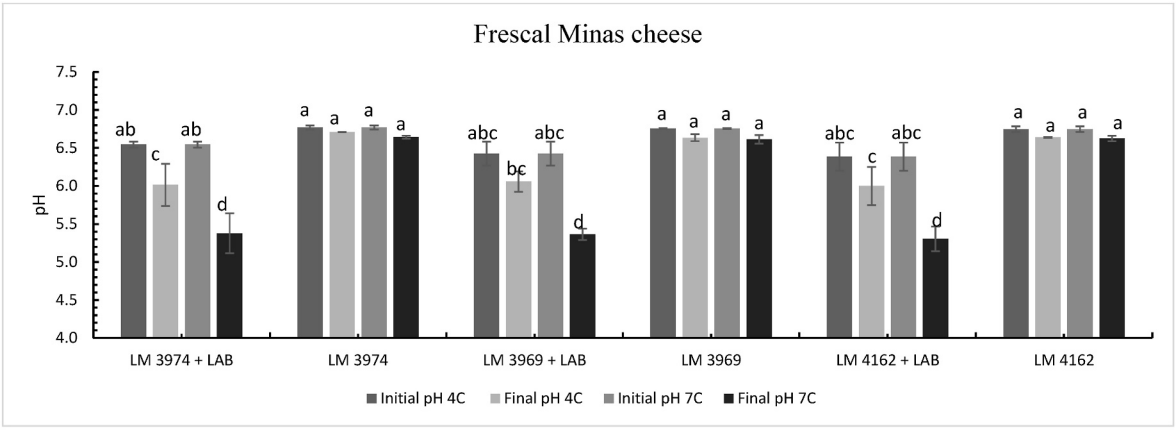
Because of the involvement of cheeses in cases of foodborne disease outbreaks (Nunes et al., 2013), several strategies have been proposed for managing cheese safety and protecting public health (D'amico, 2014). One of these strategies comprises using members of indigenous microbiota of cheeses able to inhibit or kill foodborne pathogens (Acurcio et al., 2017; Campagnollo et al., 2018, Cadavez et al., 2019, Margalho, Kamimura, et al., 2021; Pegoraro et al., 2020). The prominent indigenous members of milk and cheese with potential antimicrobial properties are lactic acid bacteria (LAB). LAB dominates the microbiota of artisanal cheeses (Kamimura, Magnani, et al., 2019, 2020; Méndez-Romero et al., 2021; Zheng et al., 2021) and can produce a range of antimicrobial compounds that impact the fate of foodborne pathogens (Pegoraro et al., 2020; Tilocca et al., 2020). However, to the best of the author's knowledge, no study has assessed the variability in the growth potential (δ) of foodborne pathogens such as *L. monocytogenes* and *S. enterica*. Therefore, in this study, the variability δ of three different strains of *L. monocytogenes* (CLIST 3974, CLIST 3969, and CLIST 4162) and *S. enterica* [*S.* Typhimurium (ATCC SM 14028), *S.* Enteritidis (SM 64), and *S.* Montevideo (SM 129)] in Frescal and semi-hard Minas cheeses added or not of LAB with antimicrobial properties and stored under different temperature conditions during shelflife or ripening period were investigated.

2. Materials and methods

2.1. Bacterial strains and inoculum preparation

L. monocytogenes (CLIST 3974, CLIST 3969, and CLIST 4162) and *S. enterica* [*S.* Typhimurium (ATCC 14028), *S.* Enteritidis (SM 64), and *S.* Montevideo (SM 129)] strains were donated by Oswaldo Cruz Foundation (Rio de Janeiro/Brazil). The six LAB strains with antimicrobial activity were *Lactobacillus brevis* 2-392, *Lactobacillus plantarum* 1–399, and four *Enterococcus faecalis* (1–37, 2–49, 2–388, and 1–400) (Campagnollo et al., 2018). Bacterial stocks were maintained in Tryptic Soy Broth (TSB; HiMedia, Mumbai, India) or de Man, Rogosa, and Sharpe (MRS; HiMedia) broth containing sterile glycerol (15 g/100 mL) at −80 °C.

L. monocytogenes and *S. enterica* cells suspensions were prepared as previously described (Sant'Ana, Franco, & Schaffner, 2012). TSB supplemented with 0.6% yeast extract (Oxoid, Basingstoke, UK) (TSB-YE) was used as a growth medium for *L. monocytogenes* strains, while strains of *S. enterica* were cultured in TSB. Final concentrations of cell suspensions of *L. monocytogenes* and *S. enterica* were adjusted at optical density 0.5 (OD630), corresponding to 108 CFU/mL, using a Beckman DU-640B UV/VIS spectrophotometer (Beckman Coulter, Brea/CA).



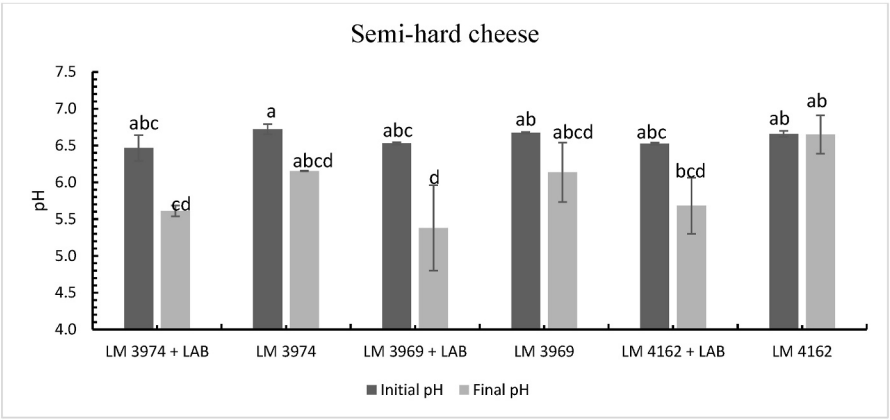


Fig. 1. Initial (day 0) and final (day 15) pH of Minas Frescal cheese (A) and ripening of semi-hard Minas cheese (day 22) (B). Cheeses were separately inoculated with three *L. monocytogenes* (LM) strains (LM 3974, LM 3969 and LM 4162) and with a pool of lactic acid bacteria (LAB) with antimicrobial activity. [a-d]Means followed by different superscript letters differ significantly according to Tukey's test ($P \leq 0.05$).

Each LAB strain was separately cultured in MRS broth, as previously detailed (Campagnollo et al., 2018). The measurement of the concentration of LAB cells was done using a McFarland scale (McFarland turbidimeter, MS Tecnopon, São Paulo/Brazil). A 1.00 reading corresponded to 3 × 108 CFU/mL. All LAB and pathogen strains were grown separately, and the cells suspensions were prepared on the day of the experiments.

2.2. Production of Frescal and semi-hard Minas microcheeses

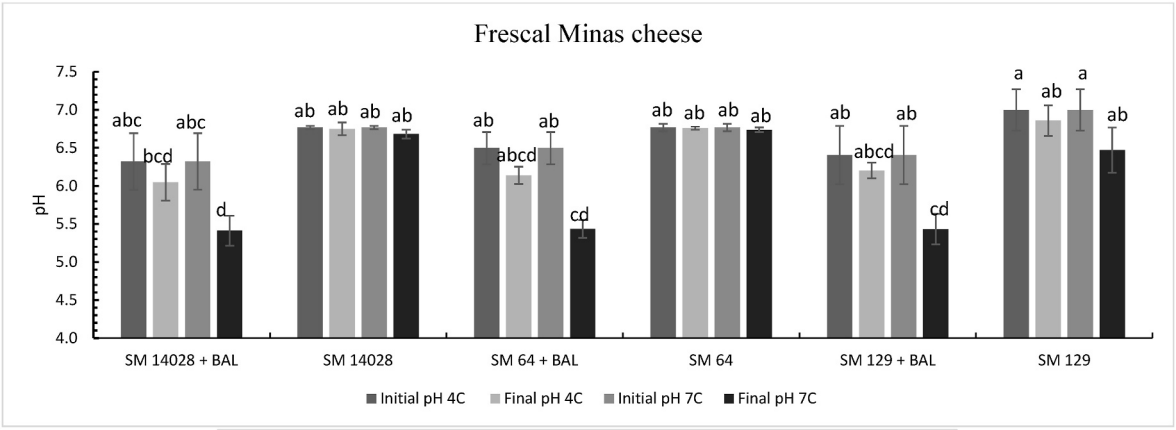
The Frescal and semi-hard Minas microcheeses were produced described as pasteurized and raw milk, respectively. The microcheeses were prepared using 250 mL of milk, yielding cheeses of approximately 25g following procedures described elsewhere (Margalho, Jorge, et al., 2021). For the production of Frescal Minas cheeses, pasteurized milk was used. Then, the drained curd was distributed into perforated sterile cylindrical shapes (4.4 × 3.3 cm), followed by dripping at room temperature and packaging the unmolded cheeses in plastic bags (Campagnollo et al., 2018), and storage at 4 and 7 °C for 15 days. For the production of semi-hard Minas cheese, raw milk was employed. After distributing curd into perforated sterile cylindrical shapes (4.4 × 3.3 cm) and dripping at room temperature, salt was added to cheese surfaces, stored on wooden shelves, and ripened at 22 °C for 22 days. During ripening, the cheeses were turned daily (Campagnollo et al., 2018).

2.3. Inoculation of LAB, L. monocytogenes and S. enterica in Frescal and semi-hard Minas microcheeses

All the Minas microcheeses were separately inoculated with each strain of *L. monocytogenes* and *S. enterica*. The inoculation of pathogens was done in the milk used for cheesemaking in a concentration of 103–104 CFU/mL for the Frescal cheese, as the growth of pathogens was expected to take place (Campagnollo et al., 2018). For the semi-hard Minas cheese, the milk was inoculated with a 105–106 CFU/mL concentration, as the inactivation of pathogens was expected to occur (Campagnollo et al., 2018). In addition, a pool of LAB with antimicrobial activity [*Lactobacillus brevis* 2-392, *Lactobacillus plantarum* 1–399, and 4 strains of *Enterococcus faecalis* (1–37, 2–49, 2–388 and 1–400)] (Campagnollo et al., 2018) in a concentration of 106–107 CFU/mL was inoculated in the milk for making of Frescal and semi-hard Minas cheeses. All microcheeses were assumed to contain naturally occurring microbiota (e.g., indigenous LAB).

2.4. Enumeration of LAB, L. monocytogenes and S. enterica in Frescal and semi-hard Minas cheeses

The enumeration of LAB, *L. monocytogenes,* and *S. enterica* was carried out at time zero (immediately after cheese production) and after 15 days of storage (4 and 7 °C) for Frescal Minas cheeses or after 22 days of ripening (22 °C) for semi-hard Minas cheeses. These enumeration points are required to calculate growth potential (δ) (Anon, 2003). The enumeration of LAB was done using MRS agar with incubation at 30 °C for 48 h (Anon, 1998). For L. *monocytogenes*, Oxford agar (OXA; HiMedia) was used with incubation at 37 °C for 48 h (Anon, 2017), while for *S. enterica*, Xylose Lysine Deoxycholate Agar (XLD; HiMedia) was used with incubation at 37 °C for 24 h (Sant'Ana, Barbosa, Destro, Landgraf, & Franco, 2012). The results were expressed as log10 CFU/g. The limit of quantification of LAB, *L. monocytogenes,* and *S. enterica* was 10^1 CFU/g.



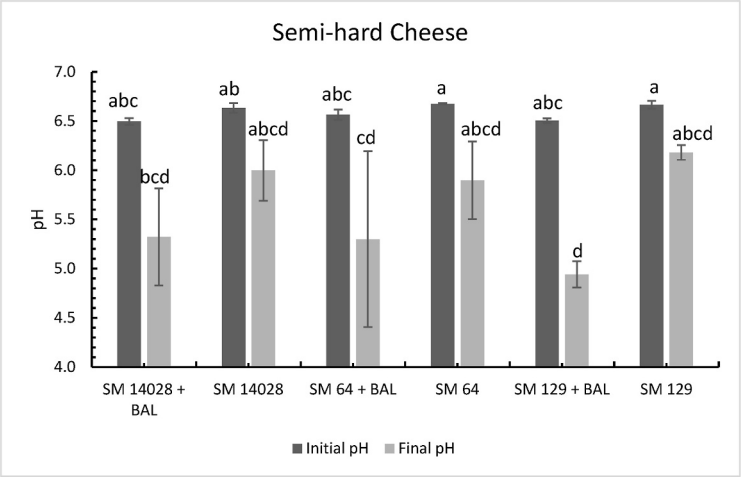


Fig. 2. Initial (day 0) and final pH after refrigerated storage of Minas Frescal cheese (15 days) (A) and ripening of semi-hard Minas cheese (22 days) (B). Cheeses were separately inoculated with three *S. enterica* (SM) strains (SM 14028, SM 64, and SM 129) and with a pool of lactic acid bacteria (LAB) with antimicrobial activity. [a-d]Means followed by different superscript letters differ significantly according to Tukey's test ($P \leq 0.05$).

2.5. pH and water activity (aw) measurements in Frescal and semi-hard Minas cheeses

The pH and aw were measured only in cheese samples inoculated with *L. monocytogenes* or *S. enterica*. pH values were determined using a portable pH meter coupled with knife electrode and temperature sensor (AK103 pHmeter, SC18 electrode, Akso Electronic Products Ltda., Rio Grande do Sul/Brazil). The aw values were determined using Aqualab CX2T (Decagon Devices Inc., Pullman/WA).

2.6. Calculation of growth potential (δ) of LAB, L. monocytogenes and S. enterica

Growth potential (δ) of LAB, *L. monocytogenes*, and *S. enterica* in each cheese was calculated by the difference between counts (in log CFU/g) at the end of storage time of the Frescal Minas cheeses (15 days) or ripening time of semi-hard Minas cheeses (22 days) and the beginning of the experiment (day zero) (Anon, 2003). The cheese supported microbial growth at a specific storage condition when δ was higher than 0.5 log10 CFU/g. When δ values were negative or lower than 0.5 log10 CFU/g, the cheese did not support microbial growth at a specific storage condition (Sant'Ana, Barbosa, et al., 2012; Sant'Ana, Landgraf, Destro, & Franco, 2013).

2.7. Statistical analyses

A total of 24 different treatments were assessed for Frescal Minas cheeses (*L. monocytogenes* or *S. enterica* × three strains × LAB addition × two storage temperatures). On the other hand, 12 treatments were assessed for semi-hard Minas cheese considering this cheese is ripened at 22 °C. All experiments were carried out in duplicate with two genuine replicates. The significant differences in counts for each microorganism over time, pH, or aw were checked using ANOVA followed by the Tukey test ($p < 0.05$). Statistical analysis and graphics were done using Microsoft® Excel 2016 (Redmond/WA).

3. Results

3.1. Growth potential (δ) of LAB, L. monocytogenes and S. enterica strains in Frescal and semi-hard Minas microcheeses

The growth potential (δ) of *L. monocytogenes, S. enterica,* and LAB after refrigerated storage of Frescal Minas and ripening of semi-hard Minas cheeses are shown in Tables 1 and 2. In Frescal Minas cheese, the addition of the pool of LAB with antimicrobial activity affected mainly the δ of LAB and *L. monocytogenes* (*Tables 1* and *2*). No significant differences in δ for LAB and *L. monocytogenes* were observed in semi-hard Minas cheese ($p < 0.05$). Furthermore, inactivation of *L. monocytogenes* strains was observed only in semi-hard Minas cheeses inoculated with LAB (Table 1).

On the other hand, the δ for LAB and *S. enterica* were not significantly different in Frescal Minas cheese no matter the storage temperature ($p < 0.05$) (Table 2). A significantly higher δ of LAB was found in semi-hard Minas cheese inoculated with *S. enterica* SM 64 ($p < 0.05$) (Table 2). Table 2 also shows that at 4 °C, the δ for *S. enterica* were always negative, indicating that under this storage temperature, inactivation took place.

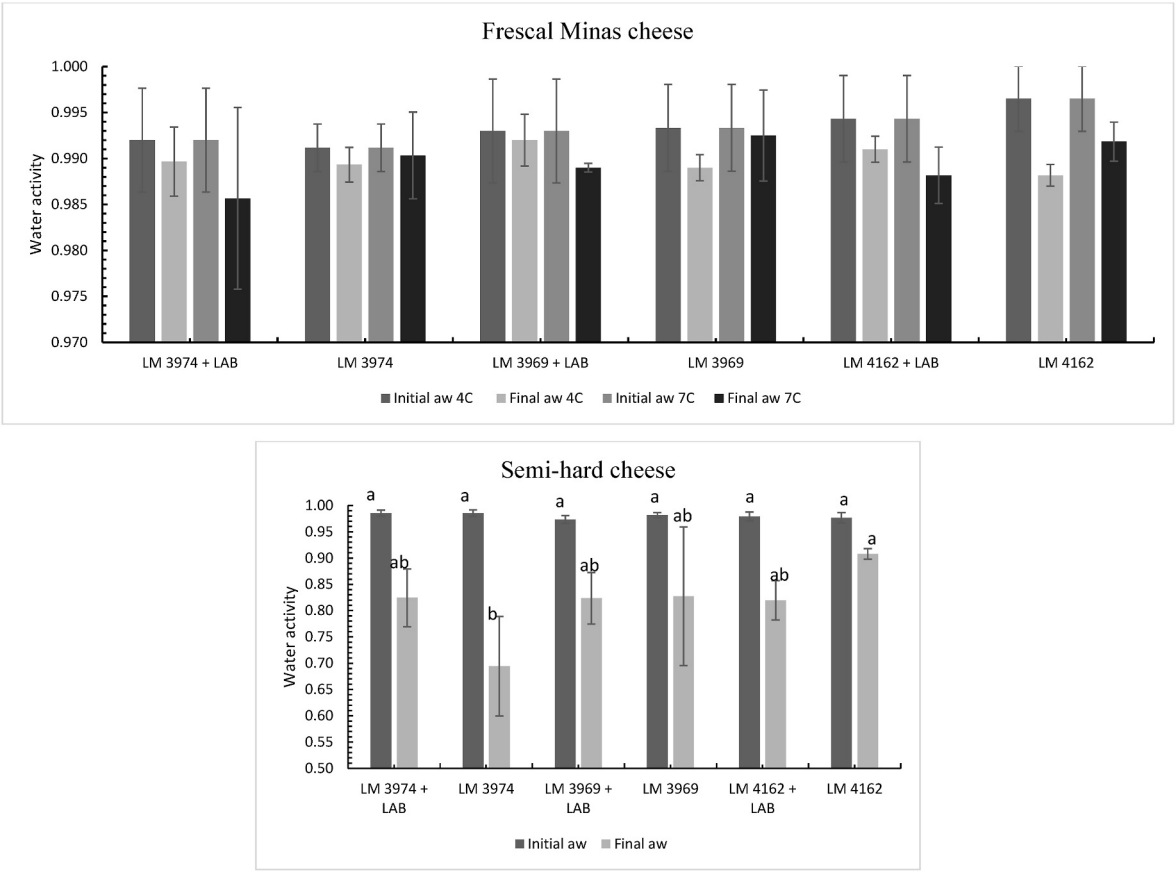


Fig. 3. Initial (day 0) and final water activity (a_w) after refrigerated storage of Minas Frescal cheese (15 days) (A) and ripening of semi-hard Minas cheese (22 days) (B). Cheeses were separately inoculated with three *L. monocytogenes* (LM) strains (LM 3974, LM 3969 and LM 4162) and with a pool of lactic acid bacteria (LAB) with antimicrobial activity. [a-d]For semi-hard cheese: means followed by different superscript letters differ significantly according to Tukey's test ($P \leq 0.05$).

On the other hand, at 7 °C, δ values for *S. enterica* were positive in most treatments (Table 2). The δ for *S. enterica* was also negative in most semi-hard Minas cheeses except for the SM 14028 and SM 129, which grew (Table 2).

3.2. Changes in pH and aw during storage of Frescal and ripening of semi-hard Minas cheeses

Initial and final pH for both types of cheese inoculated with *L. monocytogenes* and with *S. enterica* strains are shown in Fig. 1 and 2. Statistical analysis indicated a p-value <0.0001 for the cheeses inoculated with *L. monocytogenes*. When LAB with antimicrobial activity was not inoculated in Frescal Minas cheeses, no significant pH differences were observed regardless of the temperature ($p < 0.05$). However, when Frescal Minas cheeses were inoculated with LAB with antimicrobial activity, significant changes were observed in the final pH, with the highest pH reductions seen at 7 °C ($p > 0.05$). With a p-value of 0.0011, significant differences among the pH of all semi-hard Minas cheeses were seen ($P < 0.05$). The semi-hard Minas cheese inoculated with *L. monocytogenes* LM 3969 and LAB presented a pH value of 5.38, while the pH of the cheeses inoculated with the other two *L. monocytogenes* strains were 5.61 and 6.15, respectively (Fig. 1). Significant decreases in pH were mainly observed at 7 °C Frescal Minas pieces of cheese inoculated with *S. enterica* and LAB with antimicrobial activity ($p < 0.0001$) (Fig. 2). On the other hand, amongst the semi-hard Minas cheeses, the lowest pH value (4.94) was observed in the treatment inoculated with *S. enterica* SM129 and LAB with antimicrobial activity, whereas other treatments showed pH values between 5.30 and 6.18 (p-value of 0.0013) (Fig. 2).

When aw was evaluated, no significant difference amongst Frescal Minas cheeses inoculated with *L. monocytogenes* and added or not of LAB was found ($p = 0.7642$) (*Fig3*). On the other hand, significant differences were found in aw of semi-hard Minas cheeses inoculated with *L. monocytogenes* ($p = 0.0013$). Remarkably, only the cheese inoculated with *L. monocytogenes* LM 3974 with no LAB added presented differences between initial and final aw (*Fig3*)). No significant changes in aw were observed during the refrigerated storage of Frescal Minas cheese inoculated with *S. enterica* ($p = 0.8142$) (*Fig. 4*). The initial aw of semi-hard Minas cheese did not differ significantly among the treatments; however, significant variations in the final aw were found ($p = 0.0022$) (*Fig. 4*). The semi-hard Minas cheese inoculated with *S. enterica* SM 64 and LAB displayed the highest aw reduction (0.981–0.818).

4. Discussion

The δ of LAB and *L. monocytogenes* differed in Frescal Minas cheeses, with the highest δ values permanently observed when no LAB was added. On the other hand, no differences in the δ of LAB were observed in semi-hard Minas cheese, and *L. monocytogenes* was inactivated in semi-hard Minas cheese inoculated with LAB. Prior studies reported that changing over the ripening period of intrinsic factors such as higher salt content, lower pH, a_w reduction due to salt diffusion, and the competition for nutrients with the natural microbiota result in low frequency of *L. monocytogenes* in semi-hard Minas cheese (Gonzales-Barron, Campagnollo, Schaffner, Sant'Ana, & Cadavez, 2020; Kapetanakou, Gkerekou, Vitzilaiou, & Skandamis, 2017). In contrast, Frescal Minas cheese, produced with pasteurized milk, facilitates the pathogen's growth since pasteurization destroys a large part of autochthonous microbiota

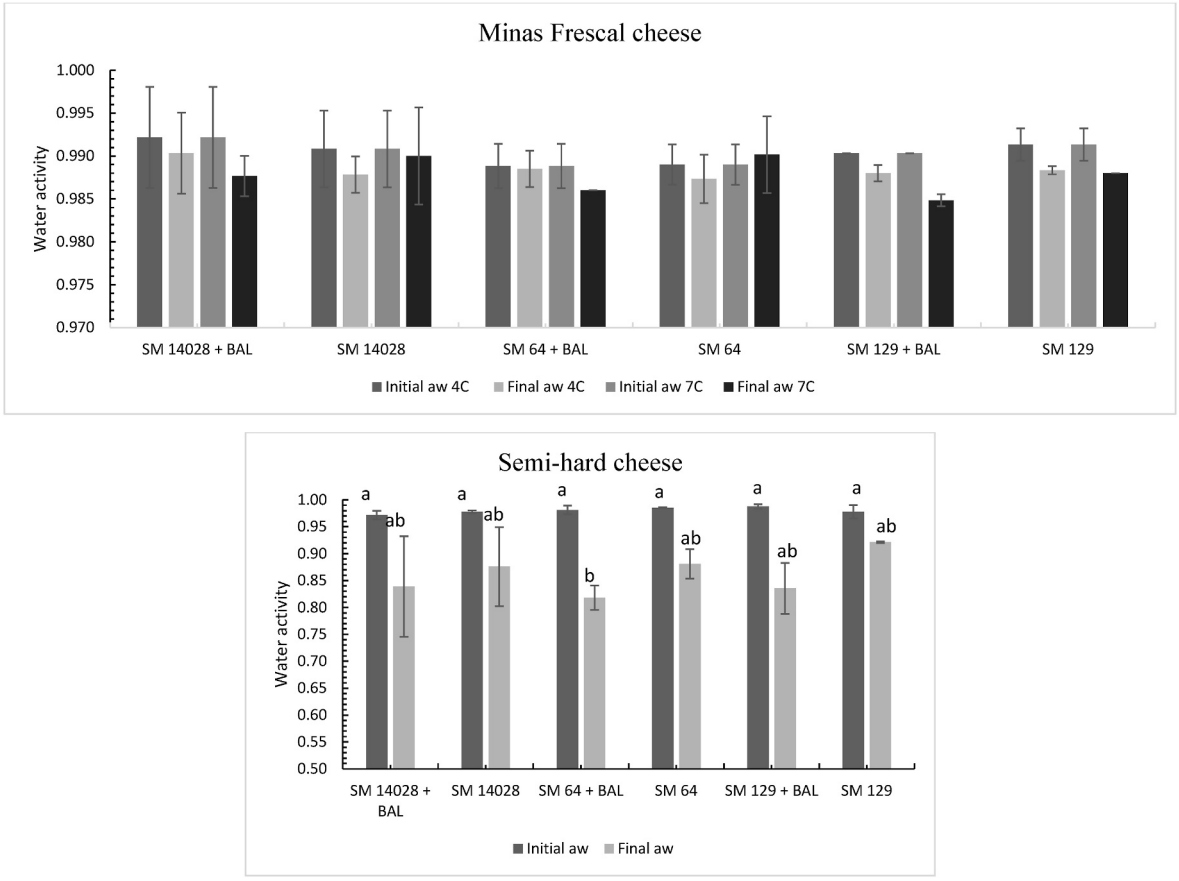


Fig. 4. Initial (day 0) and final water activity (a_w) after refrigerated storage of Minas Frescal cheese (15 days) (A) and ripening of semi-hard Minas cheese (22 days) (B). Cheeses were separately inoculated with three *S. enterica* (SM) strains (SM 14028, SM 64 and SM 129) and with a pool of lactic acid bacteria (LAB) with antimicrobial activity. [a-d]For semi-hard cheese: means followed by different superscript letters differ significantly according to Tukey's test ($P \leq 0.05$).

predominantly composed by LAB (Gérard, El-Hajjaji, Niyonzima, Daube, & Sindic, 2018). In addition, soft cheeses (such as Frescal Minas) must be stored under refrigeration, and the psychrotrophic nature of *L. monocytogenes* combined with other factors (e.g., high a_w) favors the growth of this bacterium (Campagnollo et al., 2018). The addition of LAB with antimicrobial properties decreased the δ of *L. monocytogenes* in Frescal Minas cheese and resulted in the inactivation of the pathogen in semi-hard cheese during ripening. In contrast, the δ of *L. monocytogenes* reached up to 1 log CFU/g when no LAB was inoculated. These results highlight that the use of LAB with biopreservation properties (Campagnollo et al., 2018; Costa et al., 2018) can be a feasible strategy towards enhancing the safety of these products and safeguarding public health.

LAB with antimicrobial properties influenced the final pH of cheeses they were added; however, the pH reduction varied amongst the different cheeses and treatments (cheeses inoculated with different pathogen strains). The production of organic acids through LAB metabolism depends on the pathways activated under distinct environmental conditions. A previous study reported that the undissociated lactic acid and other antimicrobial compounds produced by added LAB, which result in the inactivation of *L. monocytogenes* in cheeses, are associated with pH reduction (Wemmenhove, van Valenberg, van Hooijdonk, Wells-Bennik, & Zwietering, 2018). Overall, LAB inoculated in Frescal Minas cheese resulted in lower pH reduction, which did not lead to a considerable decrease in the δ of *L. monocytogenes*. Therefore, it seems that the addition of LAB with antimicrobial properties will be more effective in cheeses undergoing, at least, a short ripening period (~22 days). During the ripening period and conditions, these LAB with antimicrobial properties will likely outgrow and dominate the microbiota, releasing antimicrobial compounds that inhibit or inactive pathogens.

No difference was observed in the δ of LAB and *S. enterica* inoculated in Frescal Minas cheese regardless of the temperature. This finding indicates that the inoculation of LAB with antimicrobial properties did not exert an antagonistic effect on the pathogen's growth in this matrix. Interestingly, at 7 °C, the most significant pH reductions were found only in cheeses inoculated with LAB presenting antimicrobial properties. Overall, Frescal Minas cheese did not allow *S. enterica* at 4 °C, mostly due to the low storage temperature (Lobacz & Zulewska, 2021).

In semi-hard Minas cheese, the δ of LAB varied among the treatments. *S. enterica* grew in the absence of LAB with antimicrobial properties, except *S. enterica* SM 64. *S. enterica* strains were inactivated in cheeses inoculated with LAB. The lowest pH value was observed in semi-hard Minas cheese inoculated with LAB and *S. enterica* SM 129. LAB such as *Lactobacillus* sp. is known to inhibit *S.* enterica in raw milk cheese during storage at 5, 15, and 25 °C (Lobacz et al., 2020). The inactivation mechanisms of LAB against *Salmonella* are not entirely elucidated, and several extrinsic and intrinsic factors such as storage temperature, pH, composition, and autochthonous microbiota can influence the effectiveness of the produced antimicrobial compounds (Costa et al., 2019; Pegoraro et al., 2020). Bacteriocins produced by LAB are among the most potent inhibitors reported against pathogens in cheese (Lobacz & Zulewska, 2021), even though they are not known to be more effective against Gram-positive pathogens. However, other antimicrobial compounds such as hydrogen peroxide and organic acids can produce unspecific interactions, resulting in pathogen's inhibition or even inactivation (Liu, Chung, Yang, & Yousef, 2004; Yoon, Lee, & Choi, 2016). When it comes to ripened cheese, the temperature conditions in which ripening of raw milk cheese takes place has been found to

significantly influence *Salmonella* inactivation (Lobacz et al., 2020). The presence of LAB during ripening at 22 °C and the microbial competition in combination with the low pH of the cheeses made from raw milk can explain the inactivation of *S. enterica* observed in the present study.

Contrary to pH, the a_w of Frescal Minas cheeses added or not of LAB was not significantly different regardless of the pathogen inoculated. While the high a_w of Frescal Minas cheese favors *L. monocytogenes* growth (Cadavez et al., 2019), *S. enterica* was mainly inhibited mainly because of storage temperature conditions (Lobacz et al., 2020). In contrast, in semi-hard cheese, the a_w values showed differences due to ripening time, which favors reducing water content (Wemmenhove et al., 2018). The reduction in a_w can be associated with the proteolysis caused by enzymes released by LAB during the ripening period (Gonzales-Barron et al., 2020). Together with the presence of the natural microbiota, these results can explain in part the *L. monocytogenes* and *S. enterica* inactivation observed in semi-hard Minas cheese.

5. Conclusion

In this study, the effectiveness of a pool of LAB with antimicrobial properties in inhibiting *L. monocytogenes* and *S. enterica* varied with the type of cheese and strain. The addition of LAB with antimicrobial properties resulted in bacteriostatic effects and inactivation of *L. monocytogenes* in Frescal Minas cheeses and semi-hard Minas cheeses, respectively. The inactivation of *S. enterica* was significantly higher during the ripening period of semi-hard Minas cheeses produced with raw milk and added LAB. Therefore, the use of LAB strains with LAB presenting antimicrobial properties is a feasible and promising strategy to enhance the safety of soft or semi-hard cheeses. Overall, the selection and testing *in-product* of LAB to inhibit different strains of specific pathogens are essential for their successful use as biopreservation agents.

CRediT authorship contribution statement

Fernanda B. Campagnollo: Conceptualization, Methodology, Validation, Formal analysis, Writing – original draft, Visualization, Writing – review & editing. **Geany T.S. Pedrosa:** Validation, Formal analysis, Writing – original draft, Visualization, Writing – review & editing. **Bruna A. Kamimura:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing – original draft, Visualization. **Marianna M. Furtado:** Methodology, Formal analysis, Investigation, Validation, Visualization. **Rafaela C. Baptista:** Methodology, Formal analysis, Validation, Investigation, Writing – original draft, Visualization. **Henry M. Nascimento:** Methodology, Formal analysis, Validation, Investigation, Writing – original draft, Visualization. **Verônica O. Alvarenga:** Methodology, Validation, Investigation, Writing – original draft, Visualization. **Marciane Magnani:** Visualization, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. **Anderson S. Sant'Ana:** Conceptualization, Methodology, Validation, Formal analysis, Writing – original draft, Writing – review & editing, Resources, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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